2.5D Representations Combining in vivo 3D MRI and ex vivo 2D MSI Approaches to Study the Lipid Distribution in the Whole Sheep Brain

Valérie Labas, Ana Paula Teixeira-Gomes, Frédéric Andersson, Sébastien Ménigot, Martine Batailler, Hans Adriaensen, Martine Migaud, Elodie Chaillou

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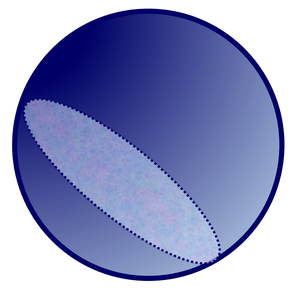
Labas V.[1-3] and Teixeira-Gomes AP.[1-4], Andersson F.[5] , Ménigot S.[5], Batailler M.[3] , Adriaensen H.[2-3], Migaud M.[3], Chaillou E.[3]

1- INRA, Plate-forme d'Analyse Intégrative des Biomolécules (PAIB2), Laboratoire de Spectrométrie de Masse, F-37380 Nouzilly, France
2- INRA, Plate-forme de Chirurgie et Imagerie pour la Recherche et l'Enseignement (CIRE), service d'imagerie in vivo, F-37380 Nouzilly, France
3- INRA, UMR INRA 85 Physiologie de la Reproduction et des Comportements, CNRS 7247, Université François Rabelais, IFCE, F-37380 Nouzilly, France
4- INRA, UMR INRA 1282 Infectiologie et Santé Publique, Université François Rabelais, F-37380 Nouzilly, France.
5- INSERM, U930 Imagerie et Cerveau, Université François Rabelais de Tours, F-37000 Tours, France

INTRODUCTION

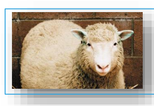
Mass Spectrometry Imaging (MSI) provides easily high spatially resolved masses allowing the characterization of endogenous lipids. These latter constitute about 70% of the composition of the white matter of the brain and are implicated in developmental and/or cognitive troubles. In order to examine the molecular distribution of lipids in whole sheep brain (gyrencephalic brain similar to human brain), we combined *in vivo* and *ex vivo* images, obtained in the same animals, using Magnetic Resonance Imaging (MRI) and MSI, respectively. In order to view the topology of the molecular species within the organ, we propose the construction of a 2.5 D representation where a single section imaged with 2D MSI is localized within the tissue volume obtained by 3D MRI. This study presents the steps required for multimodal imaging and modalities to combine *in* and *ex vivo* images with a good spatial co-registration.

2.5 D representation



PROCEEDINGS AND RESULTS

In vivo MRI



ANIMALS
2 adult sheep

Whole brain

Whole brain cryosection
Frontal and Sagittal sections (14 µm thick)

ACQUISITIONS
3D T1-weighted (w) MPRAGE images
3 T MRI scanner (Siemens Magnetom Verio®)

PROCESS
Imaging reconstruction
Osirix software

Ex vivo MSI

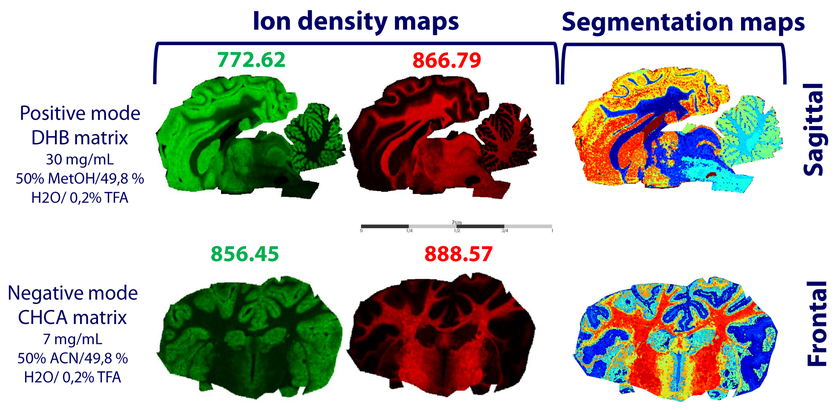
ACQUISITIONS
MALDI-TOF MS 2D imaging on lipids
UltrafleXtrem MS, ImagePrep robot (Bruker)

PROCESS
2D ion density maps and segmentation
SCiLS lab 3D software

Multimodal and correlative *in/ex vivo* imaging

MOLECULAR HISTOLOGY

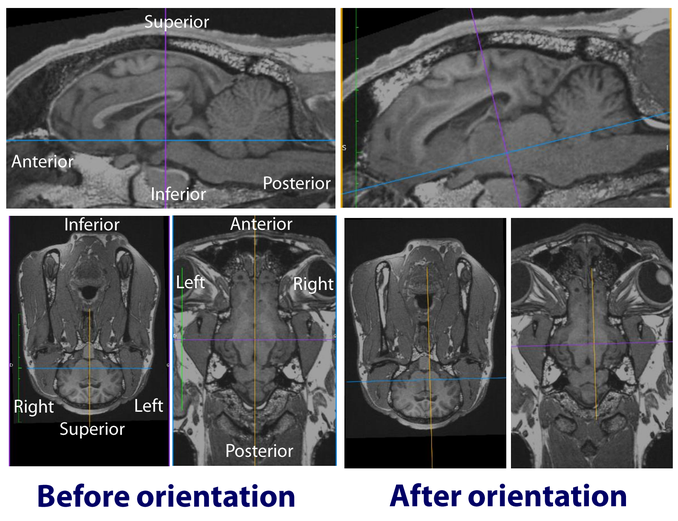
Lipidomic MSI analyses were performed in the 200–1200 m/z range with a spatial resolution set at 125 µm and treated with SCiLS Lab (TIC normalization, medium denoising), to generate 2D ion density maps (selected with ± 0,1 Da) and segmentation maps (bisecting k-Means).



From ion density or segmentation maps, frontal and sagittal sections showed a clear difference in lipid distribution, especially between grey and white matter, to the point where the cerebral cortex presents circumvolutions. Furthermore, in complement of classical cresyl violet staining, molecular histology allowed to visualize more anatomical details within the tissues.

3D REORIENTATION

The aim of this step is to find the cutting planes corresponding to the 2D histological/MSI sections within the 3D volume of the whole brain. Here, we visualize the axial, frontal and sagittal 3D T1w MR images before and after manual 3D MRI reorientation using the anatomical information. We have easily reoriented the 3D volume in order to obtain an *in vivo* plan corresponding to the *ex vivo* sagittal sections which were analyzed by MSI. However, for *ex vivo* frontal sections, we did not find the right MRI plan since each step of tissue preparation (collection, dissection, freezing, cutting) induced too strong deformations.

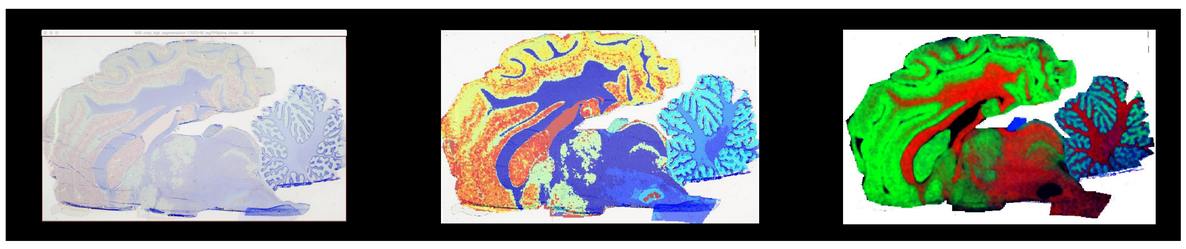


Before orientation **After orientation**

MULTIMODAL *EX VIVO* IMAGING ALIGNMENT

Cresyl violet map and MSI segmentation map were easily overlayed and co-registered (rigid transformation) using MIPAV software (Medical Image Processing and Visualization, National Institutes of Health, Bethesda, MD) and SCiLS lab software (SCiLS, germany).

In this context, SCiLS software is a tool of choice to visualize histological scans with multiple overlayed cartographies (772.62, 866.79, 872.71 m/z) in order to discover new histologic area using molecular profiles.



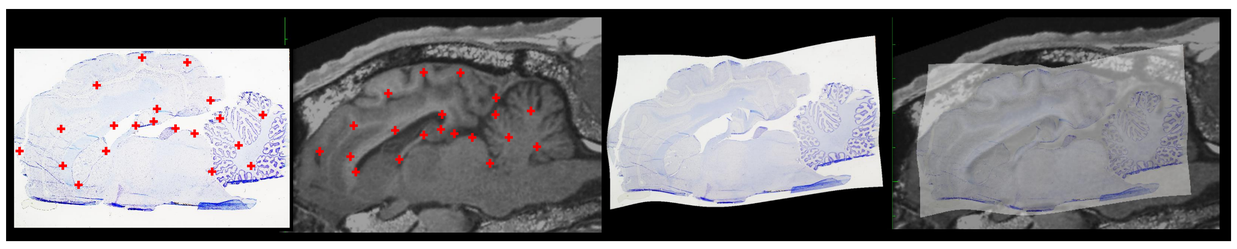
Cresyl violet + segmentation maps
MIPAV

Cresyl violet + segmentation maps
SCiLS Lab

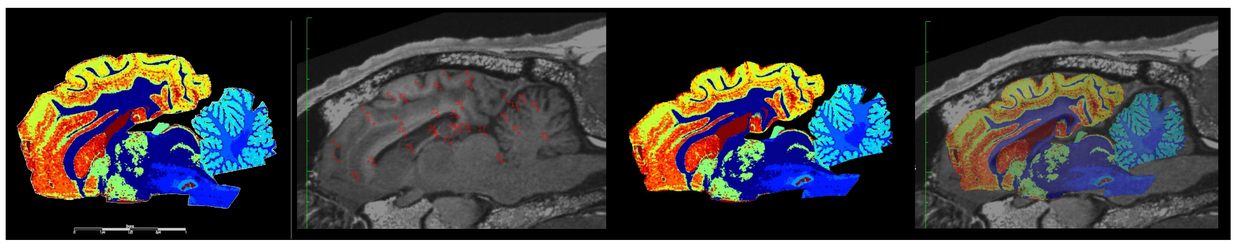
Cresyl violet + 3 ion density maps
SCiLS Lab

MULTIMODAL *IN* AND *EX VIVO* IMAGING CO-REGISTRATION

Due to the deformations induced by cutting and freezing of the organ, often encountered for large organs such as the brain, the alignment between *in vivo* (MRI) and *ex vivo* (MSI) images needed non-linear elastic co-registration. This transformation was carried out using semi-automated landmark-based method (non-linear coregistration, thin-plate-splines) available on the MIPAV software. Red crosses indicates landmarks that were choosen according to specific anatomical locations which were easily observed in all the imaging modalities : *in vivo* MRI and *ex vivo* cresyl violet or MSI segmentation map.



Cresyl violet map | MRI | Transformed Cresyl violet map | Co-registration



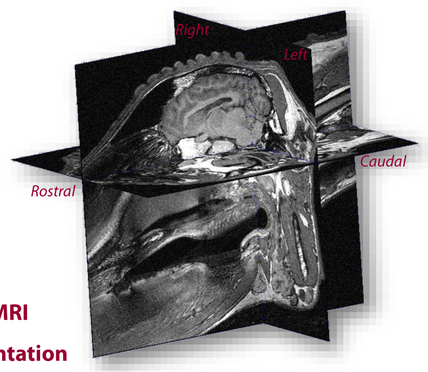
MSI segmentation map | MRI | Transformed segmentation map | Co-registration

CONCLUSION

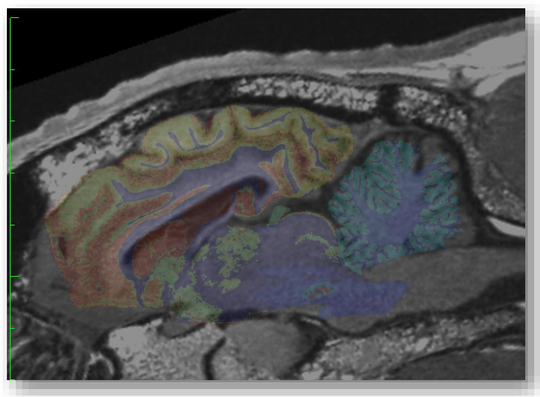
We presented a 2.5 D imaging pipeline for tissue analysis integrating three imaging modalities : (1) 3D *in vivo* Magnetic Resonance Imaging (MRI), (2) 2D *ex vivo* Cresyl violet scanning, (3) 2D *ex vivo* MALDI-TOF Mass Spectrometry Imaging (MSI) with lipidomic analysis. As a proof-of-principle-experiment, we applied the pipeline to the whole sheep brain. Here, the 2.5 D representation combining MRI and MSI (for the sagittal section) provided finer structural details on a whole large and circumvoluted brain and on the specific distribution of many lipid molecular species. We demonstrated that the 2.5 D representation is a tool of choice for exploring molecular distributions, with a perfect topology, throughout an entire complex organ such as the brain. Nowadays, the reported results may serve as a starting point for further experiments associating anatomic 3D imaging (MRI, Computerized Tomography (CT) scan) and structural 2D MSI to follow biological dynamic processes (tumors) in brain or in other organs.

2.5 D REPRESENTATION COMBINING MRI/MSI

Combined MRI and MSI is a unique approach to describe the precise distribution of each lipid species.



3D MRI representation



3D *in vivo* MRI images co-registrated with 2D *ex vivo* segmentation map obtained by lipidomic MSI

Centre de recherche Val de Loire, 37380 Nouzilly, France
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